

# Lipopolysaccharide-induced anti-inflammatory acute phase response is enhanced in spermidine/spermine $N^1$ -acetyltransferase (SSAT) overexpressing mice

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**Abstract** Bacterial lipopolysaccharide (LPS) is an effective activator of the components of innate immunity. It has been shown that polyamines and their metabolic enzymes affect the LPS-induced immune response by modulating both pro- and anti-inflammatory actions. On the other hand, LPS causes changes in cellular polyamine metabolism. In this study, the LPS-induced inflammatory response in spermidine/spermine  $N^1$ -acetyltransferase overexpressing transgenic mice (SSAT mice) was analyzed. In liver and kidneys, LPS enhanced the activity of the polyamine biosynthetic enzyme ornithine decarboxylase and increased the intracellular putrescine content in both SSAT overexpressing and wild-type mice. In survival studies, the enhanced polyamine catabolism and concomitantly altered cellular polyamine pools in SSAT mice did not affect the LPS-induced mortality of these animals. However, in the acute phase of LPS-induced inflammatory response, the serum levels of proinflammatory cytokines interleukin- $1\beta$  and interferon- $\gamma$  were significantly reduced and, on the contrary, anti-inflammatory cytokine interleukin-10 was significantly increased in the sera of SSAT mice compared with the wild-type animals. In addition,

hepatic acute-phase proteins C-reactive protein, haptoglobin and  $\alpha_1$ -acid glycoprotein were expressed in higher amounts in SSAT mice than in the wild-type animals. In summary, the study suggests that SSAT overexpression obtained in SSAT mice enhances the anti-inflammatory actions in the acute phase of LPS-induced immune response.

**Keywords** Polyamine · Spermidine/spermine  $N^1$ -acetyltransferase (SSAT) · Lipopolysaccharide (LPS) · Immune response

## Abbreviations

SSAT	Spermidine/spermine $N^1$ -acetyltransferase
ODC	Ornithine decarboxylase
LPS	Lipopolysaccharide
TNF- $\alpha$	Tumor necrosis factor alpha
NF- $\kappa$ B	Nuclear factor kappa-B
CRP	C-reactive protein
SAA	Serum amyloid A
AGP	Alpha-1-acid glycoprotein
IL-6	Interleukin-6
IL- $1\beta$	Interleukin- $1\beta$
IL-10	Interleukin-10
INF- $\gamma$	Interferon- $\gamma$
ALAT	Alanine aminotransferase

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## Introduction

Polyamines, spermidine, spermine and their diamine precursor putrescine, are cationic molecules able to interact with negatively charged macromolecules, e.g. DNA, RNA and proteins. They are essential for many cellular processes

including proliferation and differentiation and thus, their intracellular pools are tightly regulated through synthesis, degradation and membrane transport. In polyamine metabolism ornithine decarboxylase (ODC), converting ornithine to putrescine, is the rate-limiting biosynthetic enzyme while spermidine/spermine  $N^1$ -acetyltransferase (SSAT) is considered to be the key catabolic enzyme (Persson 2009). SSAT acetylates spermine and spermidine turning them suitable to polyamine oxidase-mediated degradation or excretion from the cell. Expression and activity of SSAT is induced by multiple compounds and physiological conditions. Factors affecting SSAT activity or protein amount include polyamines, polyamine analogs, toxins, drugs, hormones, cytokines, stress pathways and injuries (Pegg 2008). On the other hand, increased expression of SSAT leading to altered polyamine levels, increased oxidative damage or decreased availability of acetyl CoA and ATP, is associated with many physiological and pathophysiological processes, including carbohydrate and lipid metabolism, pancreatitis, ischemic damage and carcinogenesis (Pegg 2008).

Polyamines and SSAT also participate in processes of immune response. Infection, irritation or injury-induced immune response involves activation of diverse immune cells to produce and secrete growth factors, cytokines, chemokines and other mediators. Tumor necrosis factor alpha (TNF- $\alpha$ ) is a pleiotropic cytokine which plays a major role in inflammation by activating multiple signal transduction pathways leading to diverse biological functions (Bradley 2008). Recently, it was shown that TNF- $\alpha$  induced SSAT expression through nuclear factor kappa-B (NF- $\kappa$ B), a common mediator of diverse cellular processes including immune response (Babbar et al. 2006). NF- $\kappa$ B-induced SSAT expression results in enhanced polyamine catabolism with various consequences, e.g. decreased cell growth rate and increased apoptosis, which may be beneficial under inflammatory stress (Babbar et al. 2007). In addition, some more diverse functions of SSAT, e.g. participation in cell migration and hypoxia-induced regulation of gene expression, also affect components involved in immune response (Chen et al. 2004; de Hart et al. 2008; Baek et al. 2007). Among polyamines, spermine has been implicated as an inhibitor of inflammatory responses in several studies. For example, spermine inhibits proinflammatory cytokine synthesis in human mononuclear cells in vitro (Zhang et al. 1997). In mice, an application of spermine inhibits development of carrageenan-induced edema and partially protects against sepsis induced by cecal ligation and puncture (Oyanagui 1984; Zhu et al. 2009).

Lipopolysaccharide (LPS) is a structural constituent of the outer membrane of gram-negative bacteria. The lipid component of LPS, lipid A, is highly toxic and it is the cause of endotoxemia leading to septic shock. LPS is widely used

in animal studies to reveal the actions of immune response and components of signaling pathways. LPS is bound by a membrane receptor, toll-like receptor 4 (TLR-4), activating two principal signal transduction pathways dependent on either myeloid differentiation factor 88 (MyD88) or TIR-domain-containing adaptor inducing IFN- $\beta$  (TRIF) adaptor molecules. Both pathways activate multiple transcription factors, including NF- $\kappa$ B, through which they induce the expression of pro- and anti-inflammatory cytokines (Kumar et al. 2009). Based on its anatomical location and function, liver has an important role in detoxification of LPS. Kupffer cells in liver are induced by LPS to produce proinflammatory cytokines, including TNF- $\alpha$ , interleukin-6 (IL-6), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-18 (IL-18) and interleukin-12 (IL-12) as well as anti-inflammatory cytokine interleukin-10 (IL-10). Proinflammatory cytokines further induce hepatic natural killer cells and Th1 cells to produce interferon- $\gamma$  (INF- $\gamma$ ) (Seki et al. 2000, 2001; Jirillo et al. 2002). In hepatocytes, the cytokines and chemokines then activate the production of acute-phase proteins, including C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin and alpha-1-acid glycoprotein (AGP) (Jirillo et al. 2002; Gabay and Kushner 1999). The excess release of inflammatory mediators can result in septic shock, multiple organ failure and acute respiratory distress syndrome (Van Amersfoort et al. 2003).

Polyamines and their metabolic enzymes have been shown to affect LPS-induced immune response. The studies with SSAT-deficient mice suggest that the lack of SSAT expression results in reduced LPS-induced acute kidney injury (Zahedi et al. 2010). In mouse peritoneal macrophages exposed to LPS, spermine exerts anti-inflammatory effects by suppressing IL-12 and IFN- $\gamma$  and inducing IL-10 production (Hasko et al. 2000). On the other hand, LPS-induced ODC expression in mouse central nervous system leads to increased expression of proinflammatory molecules (Soulet and Rivest 2003).

In this study, we characterized the LPS-induced inflammatory response in mice overexpressing SSAT ubiquitously. Our results suggest that the altered polyamine metabolism of SSAT mice strengthens the anti-inflammatory response against LPS. However, the observed changes in inflammatory actions had no effect on the LPS-caused mortality of the animals.

## Materials and methods

### Materials

Three- to four-month-old SSAT overexpressing C57Bl/6JOLA<sup>Hsd</sup> mice (Pietila et al. 1997) and their wild-type littermates were used in the study. Lipopolysaccharide

(from *Escherichia coli* strain 0111:B4) was purchased from Sigma (St. Louis, MO). All animal experiments were approved by the National Animal Experiment Board.

### LPS experiments

The mice were given a single intraperitoneal injection of LPS in physiological saline. The control mice received an injection of saline. The survival experiments were done with LPS doses of 15 or 30 mg/kg. The injected mice were assessed for clinical signs (ruffled fur, diarrhea, lethargy, tremors) in every 1 or 2 h. The mice were euthanized by cervical dislocation when they reached the moribund state (unresponsive, no righting reflex). In experiments monitoring the immunological changes, the mice received LPS at doses of 2 mg/kg (non-lethal dose) or 15 mg/kg (lethal dose). Mice were killed with CO<sub>2</sub> 24 h after the non-lethal dose and at 1, 3 or 6 h after the lethal dose of LPS.

### Histology

After killing with CO<sub>2</sub>, the liver and kidney samples were immediately immersed in 4% formalin, fixed overnight at room temperature and embedded in paraffin. Ten-micrometer-thick sections were prepared and stained with hematoxylin and eosin. Histopathological analyses of inflammatory tissue response (edema, cellular necrosis, neutrophilic infiltration, hemorrhage, etc.) were made by an experienced histopathologist (RS).

### Polyamine concentrations

**ODC and SSAT enzyme activity assays** Liver and kidney were homogenized in buffer containing 25 mM Tris/HCl (pH 7.4), 0.1 mM EDTA and 0.1 M DTT. The concentration of polyamines and their acetylated derivatives were measured from tissue homogenates with high-performance liquid chromatography (Hyvonen et al. 1992). For ODC and SSAT activity assays, the homogenates were centrifuged and the assays were performed using the supernatant fraction as described previously by Jänne and Williams-Ashman (Janne and Williams-Ashman 1971) and Bernacki et al. (Bernacki et al. 1995), respectively.

### Alanine aminotransferase (ALAT) and creatinine analysis

ALAT activity and creatinine concentrations in serum were measured spectrophotometrically (Micro Lab 200, Merck) with ALAT (GPT) FS\* (IFFC mod.) kit (DiaSys, Germany) and Merckotest Creatinine kit (Merck, Darmstadt,

Germany), respectively, according to the manufacturer's instructions.

### Cytokine analysis

Cytokines IL-1 $\beta$ , IL-6, IL-10, INF- $\gamma$ , TNF- $\alpha$  were measured from serum samples using Bio-Plex Pro mouse cytokine kit, BioPlex 200 System and BioPlex Luminex XMap™ Technology (Bio-Rad, CA, USA).

### RNA analysis of acute phase proteins

Total RNA from liver homogenate was extracted by TRIzol Reagent (Invitrogen, CA, USA) and treated with DNase I (DNA-free, Ambion, CA, USA) according to the manufacturer's instructions. DNase-treated RNA was used for first-strand cDNA synthesis using random primers and AMV (avian myeloblastosis virus) reverse transcriptase (Promega, WI, USA). PCR reactions contained 1  $\mu$ l of the first-strand cDNA, 1  $\times$  PCR buffer with MgCl<sub>2</sub> (Finnzymes, Finland/MA, USA), dNTP mix (200  $\mu$ M each), 25 pmol forward and reverse primers and 0.5 units DyNAzyme DNA polymerase (Finnzymes, Finland/MA, USA) in a total volume of 50  $\mu$ l. The primers used were as listed below: Serum amyloid A (SAA), 5'-GAGCCTACACTGACATGAAG-3' (forward), 5'-TCCTCAAGCAGTTACTACTG-3' (reverse); C-reactive protein (CRP), 5'-AGCTACTCTGTGCCTTCTG-3' (forward), 5'-AGCTGCGGCTTAATAAACAC-3' (reverse);  $\alpha$ <sub>1</sub>-acid glycoprotein (AGP), 5'-CTGTCCTAAACCCTGATTAC-3' (forward), 5'-TGCTGCTTCTCCTGCTGAC-3' (reverse); Haptoglobin, 5'-TGCTGTGGAGTTGGGCAATG-3' (forward), 5'-CCCTCTGCTTGAGTTTGATTAG-3' (reverse); 18S RNA, 5'-CTACCAATCCAAGGAAG-3' (forward), 5'-CTCAGCTAAGAGCATCGAG-3' (reverse). The program consisted one cycle of 95°C for 3 min, 17 (18S RNA), 21 (Haptoglobin), 23 (CRP), 25 (Haptoglobin) or 27 (AGP1) cycles of 95°C for 30 s, 58°C for 40 s, 72°C for 40 s followed by one cycle of 72°C for 5 min. PCR products were separated on a 0.9% agarose gel and scanned using a Typhoon 9400 imager (GE Healthcare, UK). The images were analyzed using the Image Quant TL program (GE Healthcare, UK).

### Statistical analysis

Data are presented as means  $\pm$  SD when applicable. GraphPad Prism 5.03 software package (GraphPad Software Inc., LaJolla, CA) was used to perform the two-tailed Student's *t* test, 2-way ANOVA and Kaplan–Meier survival analysis. A *P* value <0.05 was considered to be statistically significant.

## Results

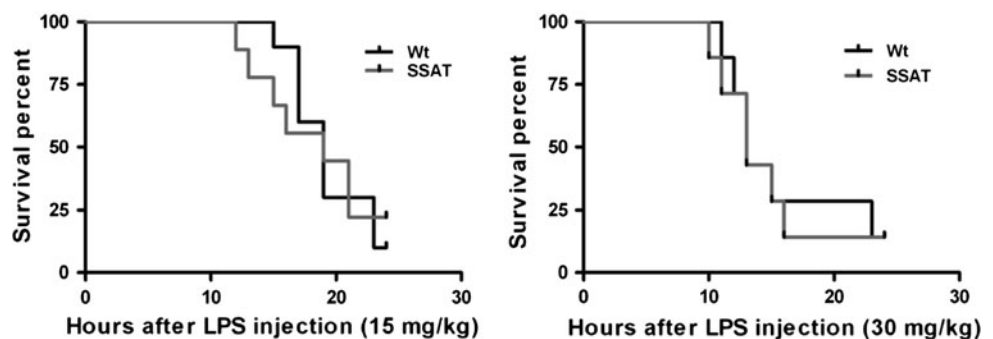
### SSAT mice and wild-type mice have similar survival rates after LPS administration

To explore the endotoxin response of SSAT mice and their wild-type littermates, the mice were subjected to 15 or 30 mg/kg of LPS and the survival of these mice was observed. There was no significant difference in the survival between the groups with either of the doses used (Fig. 1). However, SSAT mice began to develop early signs of inflammatory reaction, e.g. diarrhea and lethargy, slightly before their wild-type littermates did. To further assess the immunological changes induced by LPS, we decided to analyze the effects of a non-lethal dose of LPS within 24 h and the effects of a lethal dose of LPS within the first 6 h (acute phase).

### SSAT mice show enhanced liver response compared with wild-type mice

To assess the injurious effects of LPS on the liver, serum alanine aminotransferase activity (ALAT) and histopathological changes of liver tissue were assessed. At 24 h after non-lethal (2 mg/kg) LPS challenge, serum ALAT activities increased significantly over the basal level in both genotypes (Fig. 2a). The serum ALAT activities were significantly higher in SSAT mice in comparison with the wild-type littermates. Interestingly, treatment with the lethal dose of LPS (15 mg/kg) decreased the serum ALAT activities in the SSAT mice during the acute phase when compared with the saline-injected SSAT mice (data not shown). The serum enzyme activities of the wild-type mice stayed at the basal level after the lethal dose of LPS in the acute phase (data not shown). Histopathologically, the administration of LPS led to neutrophilic infiltration into liver sinusoids in both genotypes but no distinct tissue destruction (e.g. hepatocyte necrosis or ballooning) was observed at the light microscopic level (Fig. 3). The histopathological changes in SSAT mice were similar to that of the wild-type mice.

**Fig. 1** Survival analysis of wild-type (Wt) and SSAT overexpressing (SSAT) mice after LPS administration (15 mg/kg or 30 mg/kg) shows no difference in the mortality rate between the groups

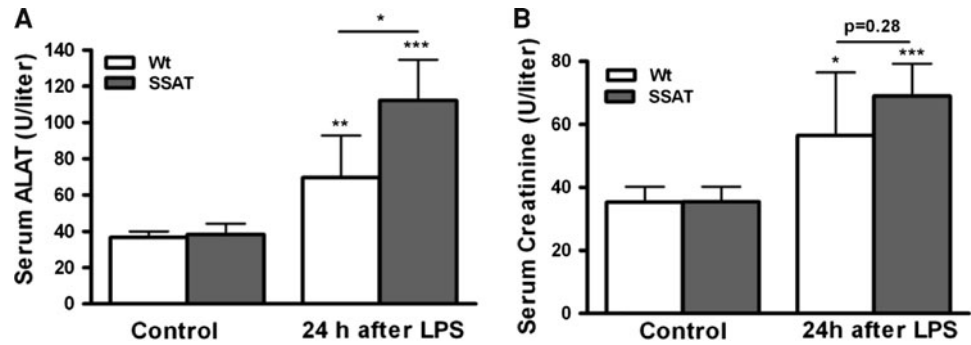


The injurious effects of LPS on the kidneys were assessed by measuring the serum creatinine levels and by evaluating the histopathological changes of the tissue. Significant increases in the serum creatinine level over the basal level were observed in both genotypes subjected to non-lethal dose of LPS (Fig. 2b). Light microscopic examination revealed no histopathological changes in the kidneys of the mice subjected to saline or LPS in either genotype (Fig. 4). Altogether, the liver and kidney of either genotype showed no tissue destruction when exposed to the non-lethal dose of LPS or during the acute phase of the LPS response. However, the response of the tissues to endotoxin was manifested by significantly increased serum ALAT activities and creatinine levels indicating submicroscopic cellular damage. Judged by ALAT activity, the liver of SSAT mice was affected to greater extent than the liver of the wild-type mice.

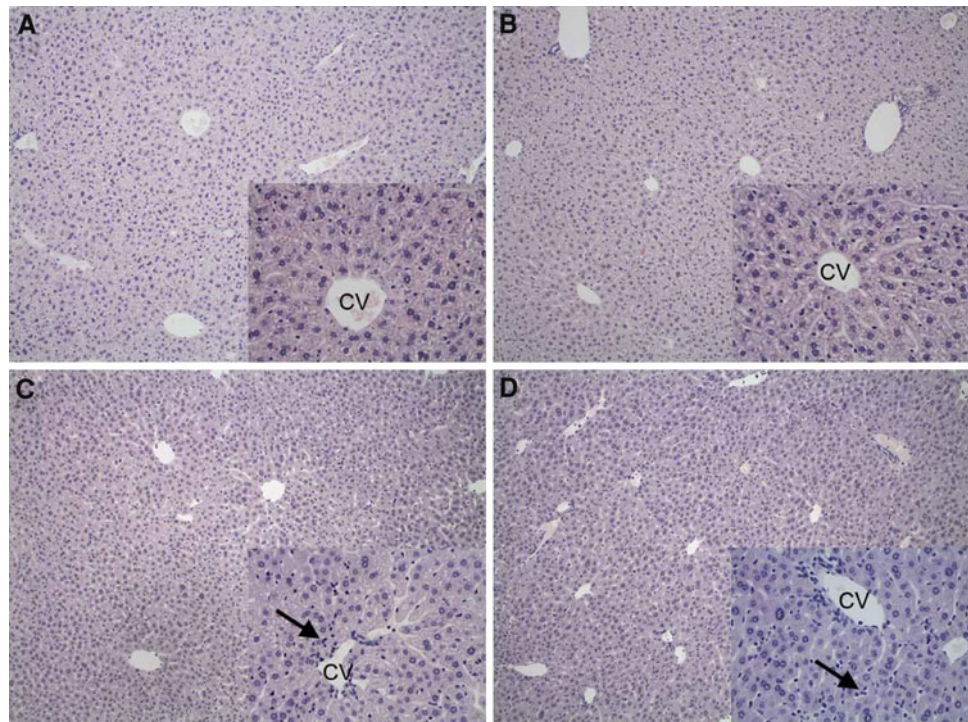
### Cytokine anti-inflammatory response to LPS is pronounced in SSAT mice

Serum concentrations of proinflammatory cytokines IL-1 $\beta$ , IL-6, TNF- $\alpha$  and INF $\gamma$  and anti-inflammatory cytokine IL-10 were determined 1, 3 and 6 h after lethal dose of LPS to elucidate the inflammatory response of the mice in more detail (Fig. 5). Serum concentrations of TNF- $\alpha$  and IL-6 were similar in SSAT and wild-type mice, TNF- $\alpha$  peaking at 1 h and IL-6 increasing rapidly within the first 6 h. The concentrations of IL-1 $\beta$  and INF $\gamma$  increased within the first 6 h after LPS administration in wild-type mice, whereas in SSAT mice, the cytokine levels increased within the first 3 h but after that reached plateau. Serum concentration of anti-inflammatory cytokine IL-10 peaked at 1 h after LPS administration in wild-type mice and after that remained elevated. In SSAT mice, the concentration of IL-10 peaked not until at 3 h after LPS challenge being significantly higher than that of wild-type mice. The level of IL-10 returned to wild-type level by 6 h after the LPS challenge. Taken together, the increase in serum concentrations of proinflammatory cytokines in SSAT mice was either similar or significantly less pronounced than in wild-type mice.

**Fig. 2** Serum alanine aminotransferase (ALAT) activities (a) and creatinine levels (b) 24 h after intraperitoneal injection of wild-type (Wt) and SSAT overexpressing (SSAT) mice with saline (control) or LPS (2 mg/kg). Data are presented as mean  $\pm$  SD ( $n = 3-5$ ). \* $p < 0.05$  \*\* $p < 0.01$ , \*\*\* $p < 0.001$



**Fig. 3** The liver samples from the wild-type (a) and SSAT overexpressing (b) mice subjected to saline show normal tissue structure. 24 h after LPS administration (2 mg/kg), there are few scattered neutrophilic leukocytes (arrows) in the sinusoids of the wild-type (c) and SSAT overexpressing (d) mice. No hepatocyte necrosis or ballooning is evident (hematoxylin-eosin staining; CV = central vein; original magnification of  $\times 100$  and in inserts  $\times 400$ )



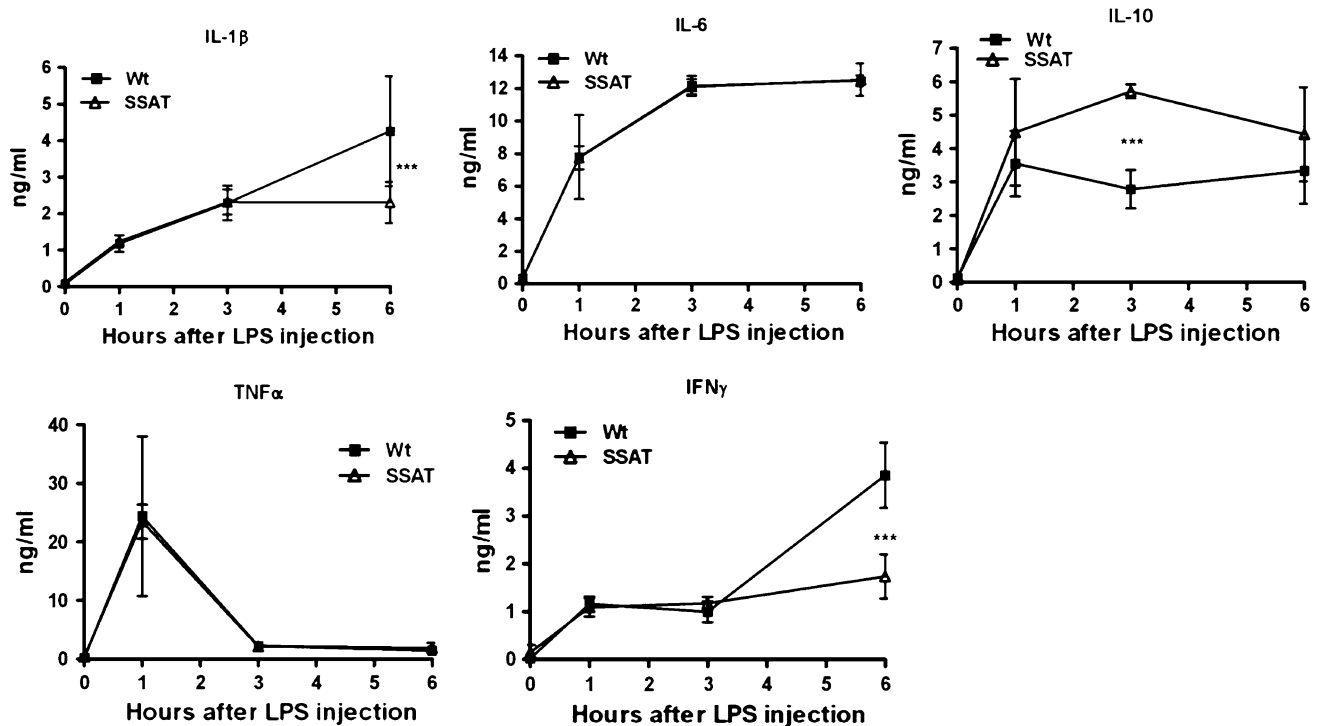
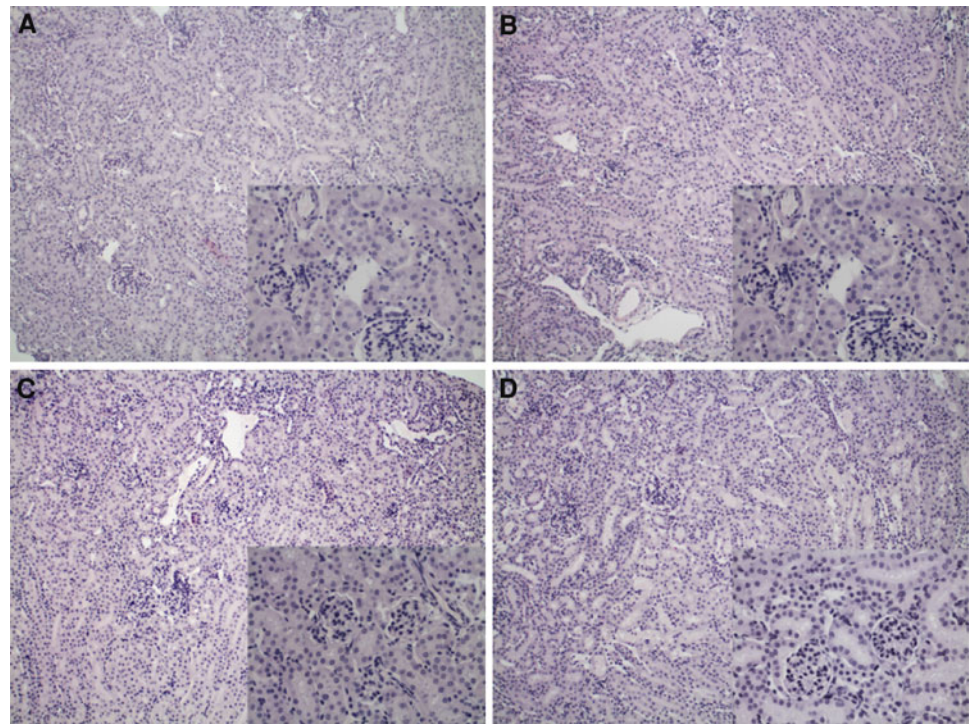
The increase in the anti-inflammatory cytokine IL-10, on the other hand, was more pronounced in SSAT mice than in the wild-type littermates.

Acute-phase protein expression in response to LPS is enhanced in SSAT mice

To further elucidate the inflammatory response of the mice, hepatic expression of acute-phase proteins CRP, SAA, haptoglobin and AGPI were quantified with RT-PCR 1, 3 and 6 h after the lethal dose of LPS (Fig. 6). The expression level of each gene was normalized to that of an internal control (18S ribosomal RNA). Wild-type and SSAT mice had similar expression levels of haptoglobin and AGPI at the basal state (0-time-point). After LPS administration, however, the expression of both genes was more pronounced in SSAT mice being significantly higher than in the wild-type mice at 3 h after the

challenge. The expression of CRP increased more slowly in SSAT mice peaking at 3 h after the LPS challenge, whereas in wild-type mice the peak was observed already at 1 h after LPS administration. Nonetheless, the highest expression level obtained in both mouse lines was similar even though the basal level in the SSAT mice was significantly lower than in the wild-type mice. In contrast to CRP, the basal expression level of SAA was higher in SSAT mice compared with that of the wild-type mice. Despite the difference in the basal level, the time course of SAA expression was parallel increasing within the first 3 h. At 6 h after LPS challenge, the expression of SAA reached a plateau in SSAT mice, while it continued to increase in the wild-type mice which leveled out the difference between the genotypes. In total, the production of acute-phase proteins CRP, AGP and haptoglobin was enhanced in SSAT mice during the acute phase of LPS response.

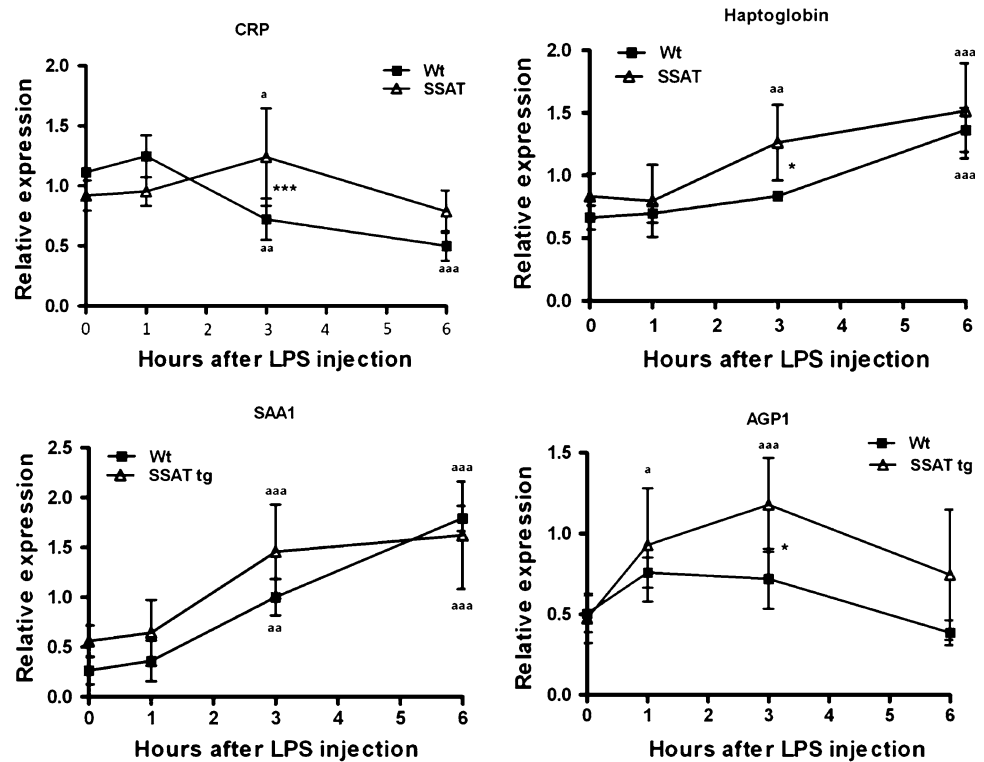
**Fig. 4** The kidney samples from the wild-type (a) and SSAT overexpressing (b) mice subjected to saline show normal tissue structure. 24 h after LPS administration (2 mg/kg), there are no histopathological changes, neither are there changes between wild-type (c) and SSAT overexpressing (d) mice. The lower magnification illustrates corticomedullary boundary. Glomeruli are normocellular with normal amount of mesangial matrix. Interstitium and tubuli are normal and there is no evidence of vasculitis (hematoxylin-eosin staining; original magnification of  $\times 100$  and in inserts  $\times 400$ )



**Fig. 5** Serum cytokine concentrations in wild-type (Wt) and SSAT overexpressing mice (SSAT) injected intraperitoneally with saline (0-time-point) or LPS (15 mg/kg). Concentrations of interleukin 1 $\beta$  (IL-1 $\beta$ ), interleukin 6 (IL-6), interleukin 10 (IL-10), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interferon  $\gamma$  (IFN $\gamma$ ) increase significantly both in

SSAT mice and in wild-type littermates after LPS challenge. Cytokine concentrations of SSAT mice were compared with those of their wild-type littermates. Data are presented as mean  $\pm$  SD ( $n = 5$ ). \* $p < 0.05$  \*\* $p < 0.01$ , \*\*\* $p < 0.001$

**Fig. 6** The expression levels of liver acute phase proteins in wild-type (Wt) and SSAT overexpressing (SSAT) mice injected intraperitoneally with saline (0-time-point) or LPS (15 mg/kg). C-reactive protein (CRP), haptoglobin, serum amyloid A (SAA) and  $\alpha_1$ -acid glycoprotein 1 (AGP1) were quantified with RT-PCR. 18S ribosomal RNA was amplified as an internal control. Expression of each gene was normalized to that of 18S RNA. Data are presented as mean  $\pm$  SD ( $n = 5$ ). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  SSAT compared with wild-type mice, <sup>a</sup> $p < 0.05$  <sup>aa</sup> $p < 0.01$ , <sup>aaa</sup> $p < 0.001$  compared with basal state (0-time-point)



Polyamine metabolic enzymes quickly respond to the lethal-dose of LPS

After the lethal dose of LPS, hepatic ODC activity increased rapidly in both genotypes (Table 1). However, the non-lethal LPS dose elevated ODC activity in SSAT mice within 24 h, whereas in wild-type mice, the activity remained at the control level (Table 2). SSAT activity increased in both genotypes after lethal dose of LPS, yet the increase was modest in wild-type mice. With non-lethal LPS dose, SSAT activity decreased significantly under the basal level within 24 h in wild-type mice. Polyamine concentrations were consistent with the detected enzyme activities (Tables 1, 2). With both lethal and non-lethal dose of LPS, putrescine accumulated in both genotypes. This was accompanied by decrease in spermidine levels in SSAT mice after lethal dose of LPS. With non-lethal dose, the spermidine level was unaltered in SSAT mice but was increased in wild-type mice. There were no changes in the concentration of spermine in either genotype after LPS administration. Similar to that of liver, kidney ODC and SSAT activities increased after lethal dose of LPS (Table 3). In contrast to liver, the enzyme activities were elevated also after non-lethal dose in SSAT mice (Table 4). Putrescine accumulated in both genotypes, accompanied by increase in spermidine level in wild-type mice (Tables 3, 4). In kidney, SSAT activity seemed to influence spermine but not spermidine levels. Taken together, compared with

each other, liver and kidney polyamine metabolism was similarly affected by LPS in both mouse lines. However, the levels of polyamines in tissues of SSAT mice and wild-type mice were markedly different both in basal and in disease state, as could be expected because of the altered polyamine catabolism in SSAT mice.

## Discussion

In this study, we used transgenic mice overexpressing SSAT under the original SSAT gene promoter. SSAT mice are characterized by several phenotypic features including hairlessness, female infertility, reduced amount of white adipose tissue, high basal metabolic rate, improved glucose tolerance, high insulin sensitivity and low plasma cholesterol level (Pietila et al. 1997; Pirinen et al. 2007, 2010). The myriad of physiological changes in these animals highlight the importance of accurately regulated polyamine metabolism. In SSAT mice, SSAT activity is increased in all tissues of the mice and the enhanced polyamine catabolism also concomitantly induces the biosynthesis seen as increased activity of ODC (Pietila et al. 1997). Increased amount of  $N^1$ -acetylspermidine and putrescine and decreased levels of spermidine and/or spermine are evident in various tissues (Pietila et al. 1997).

LPS primarily triggers the cells of innate immunity, e.g. macrophages and neutrophils. The production of

**Table 1** Liver polyamine pools in SSAT mice (SSAT) and in their wild-type (wt) littermates subjected to saline (control) or LPS (15 mg/kg)

Genotype/treatment	ODC activity (pmol/1 h per mg of protein)	SSAT activity (pmol/10 min per mg of protein)	Polyamine pools (pmol/mg of protein)			
			Putrescine	<i>N</i> <sup>1</sup> -acetylspermidine	Spermidine	Spermine
Control						
Wt	6 ± 2	2 ± 1	73 ± 98	159 ± 43	4,824 ± 761	5,174 ± 274
SSAT	50 ± 26	28 ± 9	2,558 ± 1,449	706 ± 252	65,97 ± 776	3,003 ± 554
1 h after LPS						
Wt	5 ± 2	2 ± 1	ND	ND	4,887 ± 489	5,224 ± 573
SSAT	44 ± 21	33 ± 29	2,317 ± 759	837 ± 208	6,550 ± 1482	2,799 ± 554
3 h after LPS						
Wt	164 ± 12***	4 ± 3	164 ± 72	ND	4,809 ± 838	4,952 ± 592
SSAT	393 ± 72***	110 ± 28***	2,403 ± 604	2,753 ± 667***	4,538 ± 666 **	2,633 ± 234
6 h after LPS						
Wt	362 ± 70***	5 ± 2	597 ± 308	ND	4,983 ± 622	5,267 ± 282
SSAT	568 ± 82***	57 ± 21 *	3647 ± 386	2,984 ± 1,201***	2,609 ± 1,196***	2,867 ± 252

Data are presented as mean ± SD (*n* = 5)

ND not detectable

\* *p* < 0.05 \*\* *p* < 0.01, \*\*\* *p* < 0.001 compared with control/untreated mice

**Table 2** Liver polyamine pools in SSAT mice (SSAT) and in their wild-type (wt) littermates subjected to saline (control) or LPS (2 mg/kg, killed at 24 h)

Genotype/treatment	ODC activity (pmol/1 h per mg protein)	SSAT activity (pmol/10 min per mg protein)	Polyamine pools (pmol/mg of protein)			
			Putrescine	<i>N</i> <sup>1</sup> -acetylspermidine	Spermidine	Spermine
Control						
Wt	0.4 ± 0.5	8 ± 0.1	222 ± 24	ND	6,820 ± 328	7,082 ± 618
SSAT	26 ± 11	37 ± 11	3607 ± 1,249	1,801 ± 191	8,422 ± 546	3,820 ± 180
LPS						
Wt	1 ± 1	3 ± 2*	933 ± 428*	ND	8,104 ± 507**	6,748 ± 495
SSAT	305 ± 73***	20 ± 12	11,961 ± 1,282***	1,389 ± 347	7,427 ± 630	3,509 ± 328

Data are presented as mean ± SD (*n* = 5)

ND not detectable

\* *p* < 0.05 \*\* *p* < 0.01, \*\*\* *p* < 0.001 compared with control/untreated mice

inflammatory cytokines, chemokines and, after hepatocyte activation, acute phase proteins is central for the inflammatory response. Since polyamines are known to induce, e.g. production of cytokines and recruitment of macrophages (Zhang et al. 1997; Puntambekar et al. 2011) we evaluated the effects of an altered polyamine metabolism on the LPS-induced immune response and, vice versa, the effect of LPS on polyamine metabolism in SSAT mice.

After non-lethal dose of LPS or in acute phase of the response there was no difference in the level of liver or kidney damage between SSAT mice and wild-type mice. In addition, there was no polyamine depletion in the tissues of either genotype. Several studies have shown that drastic reduction of polyamine contents, especially spermidine, leads to organ injury and/or inflammation (Alhonen et al. 2000; Rasanen et al. 2003; Rakonczay et al. 2008;

Hyvonen et al. 2010) The deleterious effects have been explained by the importance of polyamines for tissue functions and structural integrity. Thus, the lack of polyamine depletion in either mouse line may partly explain the fact that liver and kidney integrity in wild-type mice and SSAT mice was comparable. Yet, SSAT overexpression seemed to increase the incidence of submicroscopic cellular damage in liver (but not in kidneys) in the mice exposed to non-lethal dose of LPS as determined by ALAT measurements.

In our study, the dominant feature in polyamine levels after LPS exposure both in kidney and liver was the accumulation of putrescine. This was obtained by enhanced polyamine biosynthesis, since LPS increased the expression of ODC both in the wild-type and SSAT mice. ODC activity has been previously shown to be induced in mouse



**Table 3** Kidney polyamine pools in SSAT mice (SSAT) and in their wild-type (wt) littermates subjected to saline (control) or LPS (15 mg/kg)

Genotype/ treatment	ODC activity (pmol/1 h per mg of protein)	SSAT activity (pmol/10 min per mg of protein)	Polyamine pools (pmol/mg of protein)			
			Putrescine	N <sup>1</sup> -Acetylspermidine	Spermidine	Spermine
Control						
Wt	85 ± 18	33 ± 8	ND	ND	2,377 ± 68	5,030 ± 691
SSAT	385 ± 107	95 ± 16	1,132 ± 298	ND	1,612 ± 138	5,118 ± 220
1 h after LPS						
Wt	47 ± 18	53 ± 5	ND	ND	2,677 ± 334	5,418 ± 532
SSAT	357 ± 88	158 ± 25***	1,396 ± 118	335 ± 109	1,900 ± 353	4,305 ± 515*
3 h after LPS						
Wt	476 ± 82**	75 ± 11**	104 ± 89	129 ± 28	2,697 ± 265	5,332 ± 376
SSAT	950 ± 102***	150 ± 36***	1,594 ± 148**	573 ± 132	1,798 ± 146	4,310 ± 344*
6 h after LPS						
Wt	1,107 ± 104***	20 ± 2	361 ± 90	ND	2,162 ± 239	4,470 ± 680
SSAT	1,394 ± 462***	40 ± 8***	2,233 ± 301***	603 ± 179	1,430 ± 224	4,306 ± 320*

Data are presented as mean ± SD (*n* = 5)

ND not detectable

\* *p* < 0.05 \*\* *p* < 0.01, \*\*\* *p* < 0.001 compared with control/untreated mice

**Table 4** Kidney polyamine pools in SSAT mice (SSAT) and in their wild-type (wt) littermates subjected to saline (control) or LPS (2 mg/kg, killed at 24 h)

Genotype/ treatment	SSAT activity (pmol/10 min per mg protein)	Polyamine pools (pmol/mg of protein)			
		Putrescine	N <sup>1</sup> -acetylspermidine	Spermidine	Spermine
Control					
Wt	30 ± 8	442 ± 130	ND	3,158 ± 225	7,781 ± 226
SSAT	75 ± 6	2,127 ± 134	415 ± 155	2,513 ± 346	7,300 ± 639
LPS					
Wt	45 ± 10	838 ± 227*	ND	4,273 ± 291***	7,382 ± 340
SSAT	225 ± 68*	6,271 ± 643***	1,389 ± 249	2,529 ± 329	6,009 ± 933

Data are presented as mean ± SD (*n* = 5)

ND not detectable

\* *p* < 0.05 \*\* *p* < 0.01, \*\*\* *p* < 0.001 compared with control/untreated mice

brain tissue after LPS challenge (Soulet and Rivest 2003) which is in line with increased ODC activity in liver and kidneys in our study. In contrast, Zahedi et al. (Zahedi et al. 2010) detected reduced ODC activity in kidneys after LPS challenge. The induction of ODC activity and thus biosynthesis of polyamines is an important response in order to support the essential and defense-related functions of the cell under stress conditions. However, polyamine production may also contribute to the inflammatory response by modulating nitric oxide (NO) production and generation of reactive nitrogen species by reducing the availability of L-arginine, the intermediate for both polyamine and NO production (Baydoun and Morgan. 1998; Peranzoni et al. 2007).

In our study, the production of proinflammatory cytokines IL-1 $\beta$  and IFN- $\gamma$  was reduced in the acute phase of LPS-induced response in SSAT mice when compared with the wild-type mice. Interestingly, also significantly increased amount of anti-inflammatory IL-10 was seen in the sera of SSAT mice compared with the wild-type animals. IL-10 is considered to be the most important anti-inflammatory cytokine in human immune response and it is an efficient inhibitor of synthesis of Th1 cytokine IFN- $\gamma$  and monocyte/macrophage-derived cytokines including TNF- $\alpha$ , IL-1 and IL-6 (Opal and DePalo 2000). Based on that data, the cytokine profile of SSAT mice suggests increased anti-inflammatory LPS response in these animals compared with the wild-type mice. However, the reason for the observed

enhanced anti-inflammatory response is unclear. It has been shown that polyamine depletion prevents binding of NF- $\kappa$ B, the factor involved in production of many cytokines, to DNA and subsequent cytokine production of IL-8, IL-6 and MCP-1 in human chondrocytes (Facchini et al. 2005). In addition Shah et al. (Shah et al. 1999) have shown that spermine especially is a strong stimulator of NF- $\kappa$ B binding in breast cancer cells, spermidine and putrescine requiring higher concentration to reach the same effect. Spermine has also been indicated as an inhibitor of proinflammatory cytokine synthesis in human mononuclear cells in cell culture (Zhang et al. 1997).

The expression of acute-phase proteins also supports the view that SSAT overexpression enhances the anti-inflammatory actions against LPS. Acute-phase proteins are synthesized almost exclusively in the liver and are regulated mainly at the transcriptional level (Gabay and Kushner 1999; Moshage 1997). IL-1 and IL-6 synergize in induction of synthesis of acute-phase proteins SAA, CRP, AGP and haptoglobin, but also other cytokines can take over the function of these cytokines. We detected the expression of SAA, CRP, haptoglobin and AGP during acute phase of the LPS response in liver samples of both SSAT overexpressing and wild-type mice. CRP, haptoglobin and AGP showed significantly elevated expression levels in SSAT overexpressing animals compared with those in the wild-type mice. Many of the functions of acute-phase proteins are considered to be anti-inflammatory. AGP is indicated as a natural anti-inflammatory and immunomodulatory agent (Fournier et al. 2000). For example, it inhibits polymorphonuclear neutrophil activation and modulates LPS-induced cytokine secretion by monocyte/macrophages in vitro (Vasson et al. 1994; Boutten et al. 1992). In addition, AGP infusion protects mice against hepatitis and lethal shock induced by TNF- $\alpha$  (Libert et al. 1994). SAA has been indicated to have a central role in regulating anti-inflammatory response during polymicrobial sepsis by promoting myeloid-derived suppressor cell function (Sander et al. 2010). On the other hand, SAA has been shown to induce migration and adhesion of monocytes and neutrophils as well as cause neutrophilia by inhibiting neutrophil apoptosis (Badolato et al. 1994; He et al. 2009; Christenson et al. 2008). It has been shown that CRP can activate complement system and its net effect has been indicated to be anti-inflammatory, when overexpressed in transgenic mice (Gabay and Kushner 1999). Haptoglobin is characterized as an antioxidant and it protects the cells against reactive oxygen species (Gabay and Kushner 1999). For example, native haptoglobin binds neutrophils and inhibits respiratory activity of stimulated neutrophils (Oh et al. 1990).

The recent analysis of the kidney injury caused by LPS in SSAT-deficient and wild-type mice suggested that in

wild-type mice, LPS-enhanced polyamine catabolism contributed to kidney damage through generation of by-products of polyamine oxidation, e.g. hydrogen peroxide and aminoaldehydes (Zahedi et al. 2010). SSAT-deficient mice lacking the LPS-induced polyamine metabolism were protected from the tissue injury. Based on the findings in that study, increased susceptibility to LPS-induced inflammation could possibly be expected in SSAT mice. On contrary to expected increase in susceptibility to LPS, enhanced acute phase anti-inflammatory response was seen in SSAT mice. Despite the increased anti-inflammatory actions, the survival of SSAT mice after LPS-induced systemic inflammation and endotoxic shock did not, however, differ from that of the wild-type mice. There was no difference between the genotypes in the LPS-induced synthesis of TNF- $\alpha$  which is one of the common mediators of LPS signaling (Lin and Yeh 2005). In addition, the amount of TLR-4 expression in liver was not affected by SSAT overexpression (data not shown). This suggests that the main signaling events responsible for the systemic inflammation caused by LPS are not considerably affected by the altered polyamine metabolism in SSAT overexpressing animals.

We conclude that ubiquitous SSAT overexpression in SSAT mice enhances the acute-phase anti-inflammatory response against LPS. The study further emphasizes the role of polyamines and polyamine metabolic enzymes in immune response and production of inflammatory mediators. However, the detailed knowledge of the factors contributing to the enhanced anti-inflammatory response remains to be defined in the future.

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